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6/20/2003

Date

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Alan J. Schroit

Application No.: 09/974,753

Filed: October 9, 2001

For: METHODS AND COMPOSITIONS FOR
INDUCING AUTOIMMUNITY IN THE
TREATMENT OF CANCERS

Group Art Unit: 1642

Examiner: G. Nickol

Atty. Dkt. No.: UTSC:594USD1/MBW

DECLARATION OF ALAN J. SCHROIT UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Alan J. Schroit, declare that:

1. I am a citizen of the United States residing at 4619 Willow St., Bellaire, Texas 77401. I am a professor of Cancer Biology at The University of Texas, MD Anderson Cancer Center ("MD Anderson").

2. I have been employed by MD Anderson for 20 years. I have extensive experience in the field of cancer biology and, more specifically, phospholipid structures and generating antitumor

responses against tumor-expressed lipid targets through specific anti-lipid autoimmune responses. Attached as Exhibit 1 is my *curriculum vitae*.

3. In providing this declaration, I have reviewed the Office Action mailed on March 20, 2003. I am also familiar with the content of the above-captioned application, as well as with the pending claims.

4. In this Office Action, the Examiner makes reference to a publication by Bate *et al.* (1993), to dispute the patentability of claims 28-31. The Bate *et al.* reference discloses a method of making a phospholipid/polypeptide conjugate by admixing phosphatidylserine and keyhole limpet haemocyanin ("KLH") with carbodiimide. See Exhibit 2 (the Bate *et al.* reference), page 139, column 2. For the reasons set forth below, it is my opinion that the resulting phospholipid/polypeptide conjugate is not a phosphatidylserine/polypeptide conjugate. It is also my opinion that the antibodies in the antisera produced by the collaborators of the Bate *et al.* reference are not phosphatidylserine specific antibodies.

5. Phosphatidylserine is a phospholipid that has a free amine group located at the phosphate head portion of the phospholipid. It is this free amine group that distinguishes phosphatidylserine from other known phospholipids, such as phosphatidylcholine.


6. To produce the phospholipid/KLH conjugate, the collaborators in Bate *et al.* coupled phosphatidylserine to KLH by mixing KLH and phosphatidylserine in the presence of carbodiimide. See Exhibit 2, page 139, column 2. This procedure couples KLH to the phosphate

head portion of phosphatidylserine *through* the free amine group on phosphatidylserine. Because of this, the phosphatidylserine used in Bate *et al.* no longer has its distinguishing feature, the free amine group.

7. Therefore, the coupling of KLH with phosphatidylserine *via* carbodiimide does not actually produce a phosphatidylserine/KLH conjugate that preserves the phospholipids distinguishing features. It follows, then, that the antibodies produced against the conjugated product in Bate *et al.* are not specific towards phosphatidylserine.

8. I hereby declare that all statements made herein of my knowledge are true, and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the referenced patent application or any patent issued thereon.

6/19/03
Date


Alan J. Schroit, PhD

June 2003

EXHIBIT 1

CURRICULUM VITAE

NAME ALAN J. SCHROIT, Ph.D.

EDUCATION

1970 B.Sc., Microbiology and Biochemistry, Bar-Ilan University, Ramat-Gan, Israel.
1973 M.Sc., Microbiology, Hebrew University-Hadassah Medical School, Jerusalem, Israel.
1978 Ph.D., Immunology, Hebrew University-Hadassah Medical School, Jerusalem, Israel.

BACKGROUND

1996 - Adjunct Professor, Cardiovascular Research Institute, University of Limburg,
 Maastricht, The Netherlands.

1991 - Deputy Chairman, Department of Cell Biology, The University of Texas M.D.
 Anderson Cancer Center, Houston, Texas

1990 - Professor and Biologist, The University of Texas M.D. Anderson Cancer Center,
 Department of Cell Biology, Houston, Texas.

1990 - Professor of Cell Biology, Graduate School of Biomedical Sciences, The
 University of Texas Health Science Center at Houston.

1985 - 1990 Associate Professor and Associate Biologist, The University of Texas M. D.
 Anderson Cancer Center, Department of Cell Biology, Houston, Texas.

1985 - 1990 Associate Professor of Cell Biology, Graduate School of Biomedical Sciences,
 The University of Texas Health Science Center at Houston.

1983 - 1985 Assistant Professor of Cell Biology, Graduate School of Biomedical Sciences,
 University of Texas Health Science Center at Houston.

1983 - 1985 Assistant Professor and Assistant Biologist, The University of Texas System
 Cancer Center, M.D. Anderson Hospital and Tumor Institute, Department of Cell
 Biology, Houston, Texas.

1980 - 1983 Scientist, Cancer Metastasis and Treatment Laboratory, NCI, Frederick Cancer
 Research Facility, Frederick, Maryland.

1977 - 1980 Postdoctoral Fellow, Carnegie Institution of Washington, Department of
 Embryology, Baltimore, Maryland.

1971 - 1977 Research Assistant, Institute of Microbiology, Hebrew University-Hadassah
 Medical School, Jerusalem, Israel.

PROFESSIONAL SOCIETY MEMBERSHIPS

American Chemical Society

American Association for the Advancement of Science

PATENTS

U.S. Patent No. 4,571,332 "¹²⁵I-labeled Phospholipids" assigned to U.S. Government.

U.S. Patent No. 4,983,397, "Pharmaceutical Compositions Consisting of Acylated Phospholipids" assigned to Board of Regents, University of Texas System, Houston, Texas.

U.S. Patent No. 6,300,308 "Methods and Compositions for Inducing Autoimmunity in the Treatment of Cancer"

GRANT SUPPORT (Alan J. Schroit, P.I.)

NIH/NCI CA-40149 1985-1988 (approved direct costs \$168,806) Liposome Activated Macrophages for the Therapy of Metastasis.

NATO 0746/88 1989-1993 (approved direct costs 500,000 B.F.) Regulation of Transmembrane Phospholipid Distribution in Human Erythrocytes and Platelets.

NIH/NCI CA-47845 1989-1994 (approved direct costs \$741,498) Role of Phosphatidylserine in Pathology and Macrophage Recognition.

NIH/NCI DK-41714 1989-1994 (approved direct costs \$661,206) Maintenance of Lipid Asymmetry in the Human Erythrocyte.

NIH/NCI DK-41714 1995-2000 (approved direct costs \$790, 585) Maintenance of Lipid Asymmetry in the Human Erythrocyte

Texas Higher Education Board/Advanced Technology Program 1998-1999 (approved direct costs \$177,000) Induction of autoreactive lipid antibody for the recognition and destruction of metastatic tumor cells.

Elsa U. Pardee Foundation. 2001 – 2003 (\$120,000 direct costs) Antiphospholipid antibodies for cancer therapy.

NIH GM-64610 2002 - 2006 (direct costs \$660,000) Lipid peroxidation and apoptotic cell recognition.

PUBLICATIONS

1. Schroit, A.J., M. Pinkenson and A.M. Beemer. 1973. A technical aid in the cytologic diagnosis of exfoliative cells in pulmonary pathology. *Acta Cytologica* 17, 118-120.
2. Schroit, A.J., B. Geiger and R. Gallily. 1973. The capacity of macrophage components to inhibit antimacrophage serum activity. *Eur. J. Immunol.* 3, 354-359.
3. Schroit, A.J. and R. Gallily. 1974. Studies on the binding and phagocytic inhibitory properties of antimacrophage globulin (AMS). *Immunol.* 26, 971-981.
4. Gallily, R. and A.J. Schroit. 1975. Blocking of antimacrophage serum (AMS) by shedded

- macrophage antigenic components. In *Mononuclear Phagocytes in Immunity, Infection and Pathology*. (R. Van Furth, eds.) Blackwell Scientific Publications, Oxford. Pp. 363-367.
5. Schroit, A.J., S. Rottem and R. Gallily. 1976. Motion of spin-labelled fatty acids in murine macrophages Relation to cellular phagocytic activity. *Biochem. Biophys. Acta* 426, 499-512.
6. Schroit, A.J., E. Kedar and R. Gallily. 1976. A rapid and sensitive technique for the detection of Fc receptors on macrophages. *J. Immunol. Meth.* 12, 163-170.
7. Schroit, A.J. and R. Gallily. 1977. Macrophage phagocytic recognition sites Demonstration of selectivity by hetero- and alloantiserum. *Immunol.* 33, 121-127.
8. Schroit, A.J. and R. Gallily. 1977. Quantitative in vitro phagocytic rate measurements. *J. Immunol. Meth.* 17, 123-128.
9. Schroit, A.J. and R.E. Pagano. 1978. Introduction of antigenic phospholipids into the plasma membrane of mammalian cells Organization and antibody induced lipid redistribution. *Proc. Natl. Acad. Sci.* 75, 5529-5533.
10. Schroit, A.J. and R. Gallily. 1979. Macrophage fatty acid composition and phagocytosis Effect of phospholipid fatty acyl chain length and unsaturation on cellular phagocytic activity. *Immunol.* 36, 199-205.
11. Pagano, R.E., D.K. Struck and A.J. Schroit. 1980. Introduction of phospholipids into mammalian cell surfaces via lipid vesicles. In *Liposomes and Immunobiology*. (D.H. Tom and H.R. Six, eds). Elsevier, North Holland Publishing company, Amsterdam. pp. 193-209.
12. Schroit A.J. and R.E. Pagano. 1981. Capping of a phospholipid analog in the plasma membrane of lymphocytes. *Cell* 23, 105-112.
13. Pagano, R.E., O.C. Martin, A.J. Schroit, and D.K. Struck. 1981. Formation of asymmetric phospholipid membranes via spontaneous transfer of fluorescent lipid analogues between vesicle populations. *Biochemistry* 20, 4920-4927.
14. Pagano, R.E., A.J. Schroit and D.K. Struck. 1981. Interactions of liposomes with mammalian cells in vitro Studies of mechanism. In *Research monographs in cell and tissue physiology*. (J.T. Dingle and J.L. Gordon, eds.) North Holland Publishing company, Amsterdam. 7323-348.
15. Schroit, A.J. and I.J. Fidler. 1982. Effects of liposome structure and lipid composition on the activation of the tumoricidal properties of macrophages by muramyl dipeptide liposomes. *Cancer Res.* 42, 161-167.
16. Schroit, A.J. and I.J. Fidler, 1982. Stimulation of macrophage-mediated destruction of tumor cells by liposomes containing a lipophilic derivative of muramyl dipeptide. In *Current concepts in human immunology and cancer immunomodulation*. (B. Serrou et al., eds.) Elsevier Biomedical Press B.V., New York. pp. 631-637.
17. Fidler, I.J., S. Sone, W.E. Fogler, D. Smith, D.G. Braun, L. Tarcsay, R.J. Gisler, and A.J. Schroit. 1982. Efficacy of liposomes containing a lipophilic muramyl dipeptide derivative for activating the tumoricidal properties of alveolar macrophages in vivo. *J. Biol. Response Modifiers.* 1, 43-55.

18. Schroit, A.J. and I.J. Fidler. 1982. Delivery of macrophage augmenting factors encapsulated in liposomes for destruction of tumor metastases. *Prog. Clin. Biol. Res.* 102A, 347-355.
19. Schroit, A.J. 1982. Synthesis and properties of a nonexchangeable radioiodinated phospholipid. *Biochemistry.* 21, 5323-5328.
20. Fidler, I.J., W.E. Fogler, L. Tarcsay, G. Schumann, D.G. Braun and A.J. Schroit. 1983. Systemic activation of macrophages and treatment of cancer metastases by liposomes containing hydrophilic or lipophilic muramyl dipeptide. *Adv. Immunopharm.* 2, 235-241.
21. Schroit, A.J., E. Galligioni and I.J. Fidler. 1983. Factors influencing the in situ activation of macrophages by liposomes containing muramyl dipeptide. *Biol. Cell.* 47, 87-94.
22. Schroit, A.J., Hart, I.R., Madsen, J. and Fidler, I.J. 1983. Selective delivery of drugs encapsulated in liposomes Natural targeting to macrophages predicts therapeutic activity. *J. Biol. Response Modifiers.* 2, 97-100.
23. Kleinerman, E.S., Erickson, K.L., Schroit, A.J., Fogler, W.E. and Fidler, I.J. 1983. Activation of tumoricidal properties in human blood monocytes by liposomes containing lipophilic muramyl tripeptide. *Cancer Res.* 43, 2010-2014.
24. Schroit, A.J. and Key, M.E. 1983. Induction of syngeneic tumour, specific immunity by liposomes reconstituted with L2C tumour cell antigens. *Immunology.* 49, 431-438.
25. Kleinerman, E.S., Schroit, A.J., Fogler, W.E. and Fidler, I.J. 1983. Tumoricidal activity of human monocytes activated in vitro by free and Liposome-encapsulated human lymphokines. *J. Clin. Invest.* 72, 304-315.
26. Bucana, C.D., Hoyer, L.C., Schroit, A.J., Kleinerman, E. and Fidler, I.J. 1983. Ultrastructural studies of the interaction between liposome, activated human blood monocytes and allogeneic tumor cells in vitro. *Am. J. Pathol.* 112, 101-111.
27. Schroit, A.J. and Madsen, J.W. 1983. Synthesis and properties of radioiodinated phospholipid analogs that spontaneously undergo vesicle-vesicle and vesicle-cell transfer. *Biochemistry.* 22, 3617-3623.
28. Tanaka, Y. and Schroit, A.J. 1983. Insertion of fluorescent phosphatidylserine into the plasma membrane of red blood cells Recognition by autologous macrophages. *J. Biol. Chem.* 258, 11335-11343.
29. Fidler, I.J. and Schroit, A.J. 1984. Synergism between lymphokines and muramyl dipeptide encapsulated in liposomes In situ Activation of macrophages and therapy of spontaneous metastasis. *J. Immunol* 133, 515-518.
30. Schroit, A.J., Tanaka, Y., Madsen, J. and Fidler, I.J. 1984. The recognition of red blood cells by macrophages Role of phosphatidylserine and possible implications of membrane phospholipid asymmetry. *Biol. Cell* 51, 227-238.
31. Schroit, A.J., Madsen, J. and Tanaka, Y. 1985. In vivo recognition and clearance of red blood cells containing phosphatidylserine in their plasma membranes. *J. Biol. Chem.* 260, 5131-5138.
32. Schroit, A.J. and Fidler, I.J. 1985. The use of activated macrophages for the destruction of heterogenous metastasis in "Mechanisms of Metastasis", (Honn, K.V., Powers, W.E. & Sloane, B.F. (eds)). Martinus Nijhoff Pub., Boston Ch. 14, Pp. 207-226.

33. Schwartz, R.S., Tanaka, Y., Fidler, I.J., Chiu, D., Lubin, B. and Schroit, A.J. 1985. Increased adherence of sickled and phosphatidylserine enriched human erythrocytes to cultured human peripheral blood monocytes. *J. Clin. Invest.* 75, 1965-1973.
34. Nayar, R. and Schroit, A.J. 1985. Generation of pH sensitive liposomes Use of large unilamellar vesicles containing N-succinyl-phosphatidylethanolamine. *Biochemistry.* 24, 5967-5971.
35. Fidler, I.J. and Schroit, A.J. 1986. Macrophage recognition of self from non-self Implication for the interaction of macrophages with neoplastic cells. In *Symposium on Fundamental Cancer Research.* (M. Kripke and P. Frost, eds.) Raven Press, New York. Vol. 38, Pp. 183-207.
36. Tanaka, Y. and Schroit, A.J. 1986. Calcium-phosphate-induced immobilization of fluorescent phosphatidylserine in synthetic bilayer membranes Inhibition of lipid transfer between vesicles. *Biochemistry.* 25, 2141-2148.
37. Ratner, S., Schroit, A.J., Vinson, S.B. and Fidler, I.J. 1986. Analogous recognition of membrane phospholipids by insect phagocytes and mammalian macrophages. *Proc. Soc. Exptl. Biol.* 182, 272-276.
38. Schroit, A.J. and Fidler, I.J. 1986. In situ activation of macrophages for the destruction of metastasis. In *Neoadjuvant Chemotherapy,* (C. Jacquillat, M. Weil, and D. Khayat, eds.) Colloque Inserm, John Libbey Eurotext, London, 137, 121-130.
39. Schroit, A.J. and Fidler, I.J. 1986. The design of liposomes for delivery of immunomodulators to host defense cells. In *medical application of liposomes,* (Yagi, K. ed.) Japan Scientific Societies Press; Karger, Tokyo. Pp. 141-149.
40. Nayar, R., Schroit, A.J. and Fidler, I.J. 1986. Liposome encapsulation of muramyl peptides for activation of macrophage cytotoxic properties. *Methods in Enzymology* 132, 594-603.
41. Schroit, A.J., Madsen, J. and Nayar, R. 1986. Liposome-cell interactions In vitro discrimination of uptake mechanism and in vivo targeting strategies to mononuclear phagocytes. *Chem. Phys. Lipids* 40, 373-393.
42. Denkins, Y. and Schroit, A.J. 1986. Phosphatidylserine decarboxylase Generation and determination of the asymmetric distribution of phosphatidylserine in model membrane systems. *Biochim. Biophys. Acta* 862, 343-351.
43. Schroit A.J., Madsen J. and Ruoho, A.E. 1987. Radioiodinated, photoaffinity)labeled phosphatidylcholine and phosphatidylserine Transfer properties and differential photoreactive reaction with human erythrocyte membrane proteins. *Biochemistry* 26, 1812-1819.
44. Connor, J. and Schroit, A.J. 1987. Red Blood Cell Recognition by the Reticuloendothelial System. In *Red Blood Cell Carriers Potential Therapeutic Applications* (C. Ropars, ed.). *Advances in the Biosciences*, Vol. 67, Pergamon Press, pp. 163-171.
45. Connor, J. and Schroit, A.J. 1987. Determination of Lipid Asymmetry in Human Red Cells by Resonance Energy Transfer. *Biochemistry*, 26, 5099-5105.
46. Fidler, I.J., Nayar R. and Schroit, A.J. 1988. Systemic macrophage activation with liposome entrapped immunomodulators. In *Liposomes as drug carriers Trends and progress* (G. Gregoriadis, ed.), John Wiley & Sons, London, 115-129.

47. Nayar, R., Fidler, I.J. and Schroit, A.J. 1988. Potential applications of pH-sensitive liposomes as drug delivery systems. In *Liposomes as drug carriers Trends and progress* (G. Gregoriadis, ed.), John Wiley & Sons, London, 771-782.
48. Nayar, R., Tilcock, C.P.S., Hope, M.J., Cullis, P.R. and Schroit, A.J. 1988. N-succinyldioleoylphosphatidylethanolamine Structural preferences in pure and mixed model membranes. *Biochim. Biophys. Acta*, 9, 731-41.
49. Connor, J. and Schroit, A.J. 1988. Transbilayer movement of phosphatidylserine in erythrocytes Inhibition of transport and preferential labeling of a 31,000 Dalton protein by sulfhydryl reactive reagents. *Biochemistry*, 27, 848-851.
50. Fidler, I.J. and Schroit, A.J. 1988. Recognition and destruction of neoplastic cells by activated macrophages Discrimination of altered self. *Biochim. Biophys. Acta*. 948, 151-173.
51. Nayar, R. and Schroit, A.J. 1989. Ph-sensitive liposomes for the delivery of immunomodulators. In *Liposomes in the therapy of infectious diseases and cancer*, I.J. Fidler and G. Lopez-Berestein (eds.). New York Alan Liss, Inc. pp. 427-439.
52. Connor, J., Bucana, C., Fidler, I.J. and Schroit, A.J. 1989. Differentiation-dependent expression of phosphatidylserine in mammalian plasma membranes Quantitative assessment of outer leaflet lipid by prothrombinase complex formation. *Proc. Natl. Acad. Sci. (USA)* 86, 3184-3188.
53. Madsen, J., Connor, J. and Schroit, A.J. 1989. Recognition of phosphatidylserine by the reticulendothelial system. In *Phospholipid Research and the Nervous System. Biochemical and Molecular Pathology* (N.G. Bazan, L.A. Horrocks and Toffano G, eds.). Fidia Research Series, Vol. 17, Springer Verlag, New York, pp. 3-10.
54. Schroit, A.J. and Connor, J. 1989. Transbilayer movements of phosphatidylserine in Erythrocytes. In *Phospholipid Research and the Nervous System. Biochemical and Molecular Pathology* (L. Freyz, Hawthorne, J.N. and Toffano G., eds.). Fidia Research Series, Vol. 20, Springer Verlag, New York, pp. 48-55.
55. Connor, J. and Schroit, A.J. 1989. Transbilayer movement of phosphatidylserine in non-human erythrocytes Evidence that the aminophospholipid transporter is a ubiquitous membrane protein. *Biochemistry*, 28, 9680-9685.
56. Connor, J. and Schroit, A.J. 1990. Aminophospholipid translocation in erythrocytes Evidence for the involvement of a specific transporter and an endofacial protein. *Biochemistry* 29, 37-43.
57. Connor, J. and Schroit, A.J. 1990. Transport of Phosphatidylserine Across Erythrocyte Membranes" in "Cellular and Molecular Biology of Normal and Abnormal Erythroid Membranes." Cohen, C.M. and Palek, J., eds, UCLA Symposia on Molecular and Cellular Pathology, New Series, Vol. 118, Alan R. Liss, Inc., New York, pp. 161-170.
58. Connor, J., Gillum, K. and Schroit, A.J. 1990. Maintenance of lipid asymmetry in red blood cells and ghosts Effect of divalent cations and serum albumin on the transbilayer distribution of phosphatidylserine. *Biochim. Biophys. Acta* 1025, 82-86.
59. Comfurius, P., Senden, J.M.G., Tilly, R.H.J., Schroit, A.J., Bevers, E.M. and Zwaal, R.F.A. 1990. Loss of membrane phospholipid asymmetry in platelets and red cells may be

- associated with calcium-induced shedding of plasma membrane and inhibition of aminophospholipid translocase. *Biochim. Biophys. Acta* 1026, 153-160.
60. Schroit, A.J., Bloy, C., Connor, J. and Cartron, J.-P. 1990. Involvement of Rh Blood Group Polypeptides in the Maintenance of Aminophospholipid Asymmetry. *Biochemistry* 29, 10303-10306.
 61. Connor, J. and Schroit, A.J. 1991. Transbilayer movement of phosphatidylserine in erythrocytes: Inhibitors of aminophospholipid transport block the association of photolabeled lipid to its transporter. *Biochim. Biophys. Acta* 1066, 37-42.
 62. Utsugi, T., Schroit, A.J., Connor, J., Bucana, C.D. and Fidler, I.J. 1991. Elevated expression of phosphatidylserine in the outer membrane leaflet of human tumor cells and recognition by activated human blood monocytes. *Cancer Res.* 51, 3062-3066.
 63. Schroit, A.J. and Zwaal, R.F.A. (1991). Transbilayer movement of phospholipids in red cell and platelet membranes. *Biochim. Biophys. Acta* 1071, 313-329.
 64. Connor, J., Pak, C.H., Zwaal, R.F.A. and Schroit, A.J. (1992) Bidirectional transbilayer movement of phospholipid analogs in human red blood cells. *J. Biol. Chem.* 267, 19412-19417.
 65. Connor, J., Bar-Eli, M., Gillum, K.D. and Schroit, A.J. (1992) Evidence for a Structurally Homologous Rh-like Polypeptide in Rh_{null} Erythrocytes. *J. Biol. Chem.* 267, 26050-26055.
 66. Coderre, P. and Schroit, A.J. (1993) The Aminophospholipid Transporter from Human Red Blood Cells. In *Phospholipids and Signal Transduction* (Massarelli, R., Horrocks, L.A., Kanfer, J.N. and Lofferholz, K. eds.). NATO ASI Series Vol H 70, Springer Verlag, New York.
 67. Schroit, A.J. (1994) Protein-Mediated Phospholipid Movement in Red Blood Cells. in: *Current Topics in Membranes* (Hoekstra, D., ed.) Academic Press, New York. 40, 47-74.
 68. Connor, J., Pak, C.C., and Schroit, A. J. (1994) Exposure of Phosphatidylserine in the Outer Leaflet of Human Red Blood Cells: Relationship to Cell Density, Cell Age, and Clearance by Mononuclear Cells. *J. Biol. Chem.* 269, 2399-2404.
 69. Schroit, A.J. (1995) The erythrocyte aminophospholipid translocase. In *Trafficking of Intracellular Membranes: From molecular sorting to membrane fusion.* (Pedroso De Lima, M., Duzgunes, N., and Hoekstra, D. eds), NATO ASI series, Springer-Verlag, New York. 91, 35-48
 70. Bruckheimer, E. B., Gillum, K. D. and Schroit, A. J. (1995) Co-localization of Rh polypeptides and the aminophospholipid transporter in dilauroylphosphatidylcholine-induced erythrocyte vesicles. *Biochim. Biophys. Acta.* 1235, 147-154.
 71. Bevers, E.M., Wiedmer, T., Comfurius, P., Zhao, J., Smeets, E.F., Schlegel, R.A., Schroit, A.J., Weiss, H.J., Williamson, P., Zwaal, R.F.A., and Sims, P.J. (1995) The complex of phosphatidylinositol 4,5-bisphosphate and calcium ions is not responsible for Ca⁺²-induced loss of phospholipid asymmetry in the human erythrocyte: A study in Scott Syndrome, a disorder in calcium-induced phospholipid scrambling. *Blood* 86, 1983-1991..
 72. Bruckheimer, E.B. and Schroit, A.J. (1996) Membrane Phospholipid Asymmetry: Host

- Response to the Externalization of Phosphatidylserine. *J. Leukocyte Biol.* 59, 784-788.
73. Diaz, C., Morkowski, J and Schroit A.J. (1996) Generation of phenotypically aged phosphatidylserine-expressing erythrocytes by dilauroylphosphatidylcholine-induced vesiculation. *Blood* 87, 2956-2961.
74. Diaz, C., and Schroit A.J. (1996) Role of Translocases in the Generation of Phosphatidylserine Asymmetry. *J. Memb. Biol.* 151, 1-9.
75. Zwaal, R.F.A., and Schroit, A.J. (1997) Pathophysiological Implications of Membrane Phospholipid Asymmetry. *Blood* 89, 1121-1132.
76. Balasubramanian, K., Chandra, J., and Schroit, A.J. (1997) "Immune clearance of PS-expressing cells by phagocytes: The role of β_2 -glycoprotein I in macrophage recognition. *J. Biol. Chem.* 272, 31113-31117
77. Diaz, C., K. Balasubramanian, and Schroit A. J. (1998) Synthesis of disulfide-containing phospholipid analogs for the preparation of head-group specific lipid antigens. *Bioconj. Chem.* 9, 250-254.
78. Balasubramanian, K. Killion, J.J. and Schroit AJ. (1998) Estimation of plasma beta 2-glycoprotein I levels by competitive ELISA. *Thrombosis Res.* 92, 91-97.
79. Balasubramanian, K. K. and Schroit, A.J. (1998) Characterization of phosphatidylserine-dependent β_2 -glycoprotein I macrophage interactions: Implications for apoptotic cell clearance by phagocytes. *J. Biol. Chem.* 273, 29272-29277.
80. Dekker, D.W.C., Comfurius, C., Schroit, A.J., Bevers, E.M.. and Zwaal, R.F.A. (1998). Transbilayer movement of NBD-labeled phospholipids in blood cell membranes: Outward directed transport by the Multidrug Resistance associated Protein (MRP1). *Biochemistry* 37, 14833-14837.
81. Diaz, C., Lee, T. A., McConkey, D.J. and Schroit A.J. (1999) Phosphatidylserine externalization during differentiation-triggered apoptosis of erythroleukemic cells. *Cell Death and Diff.* 6, 218-226.
82. Killion, J.J., and Schroit, A.J. (1999) Cell membrane lipids as experimental therapeutic targets. *Emerging Therapeutic targets.* 3, 453-468. Ashley Pub., London, England
83. Dombroski, D, Balasubramanian, K., and Schroit, A.J. (2000) Binding of β_2 -glycoprotein I to Phosphatidylserine-Expressing Endothelial Cells Promotes "Phospholipid" Antibody-Induced Thrombosis. *J. Autoimmunity* 14, 221-229.
83. Lee, A.T., Balasubramanian, K, and Schroit, A. J. (2000) β_2 -Glycoprotein I-dependent alterations in membrane properties. *Biochim. Biophys. Acta.* 1509, 475-484.
84. Diaz C. Leon G. Rucavado A. Rojas N. Schroit AJ. Gutierrez JM. (2001) Modulation of the susceptibility of human erythrocytes to snake venom myotoxic phospholipases A(2): Role of negatively charged phospholipids as potential membrane binding sites. *Arch. Biochem. Biophys.* 391:56-64.
85. Balasubramanian K. Bevers EM. Willems GM. Schroit AJ. (2001) Binding of annexin V to membrane products of lipid peroxidation. *Biochemistry.* 40:8672-8676.
86. Balasubramanian, K. and Schroit, A.J. (2003) Aminophospholipid asymmetry: A matter

- of life and death. *Annu. Rev. Physiol.* 65:701-734.
87. Serinkan, F.B., Tyurina Y.Y., Babu, H., Djukic, M., Schroit, A.J. and Kagan, V.E. (submitted). Vitamin E inhibits anti-Fas induced phosphatidylserine oxidation but does not effect its externalization during apoptosis in Jurkat T cells and their phagocytosis by J774A.1 macrophages.
 88. Tyurina, Y.Y., Serinkan, F.B., tyurin, V.A., Kini, V., Yalowich J.C., Schroit, A.J., Fadeel, B. and Kagan, V.E. (submitted) Etoposide inhibits phosphatidylserine externalization by blocking its oxidation during apoptosis in HL-60 cells. Antioxidant dissociation of phosphatidylserine signaling from the final common pathway for apoptosis.